Supporting Information

Compartmental Genomics in Living Cells Revealed by Single-Cell Nanobiopsy

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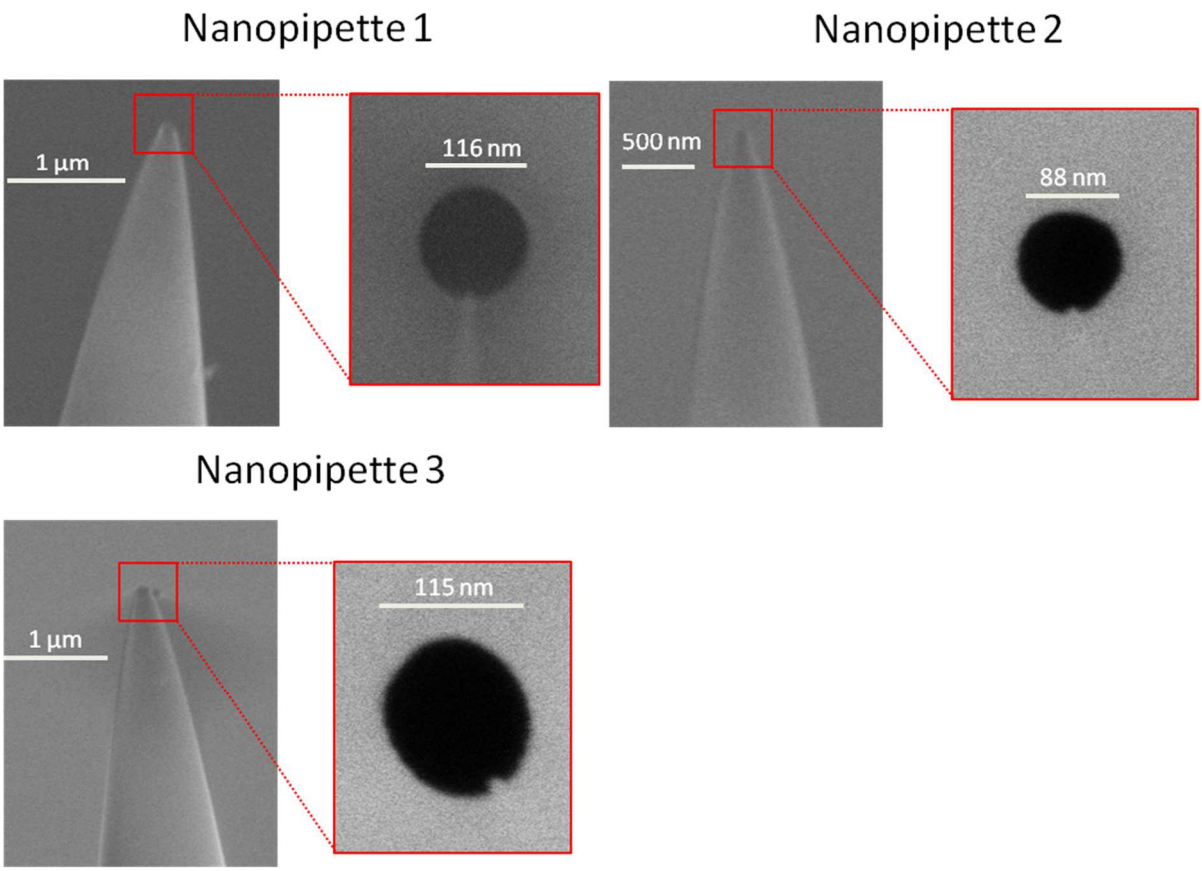
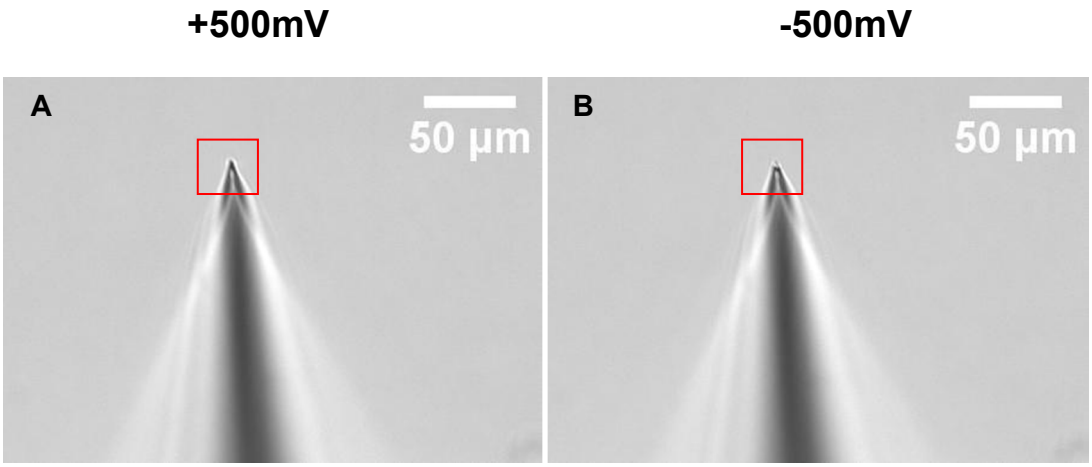
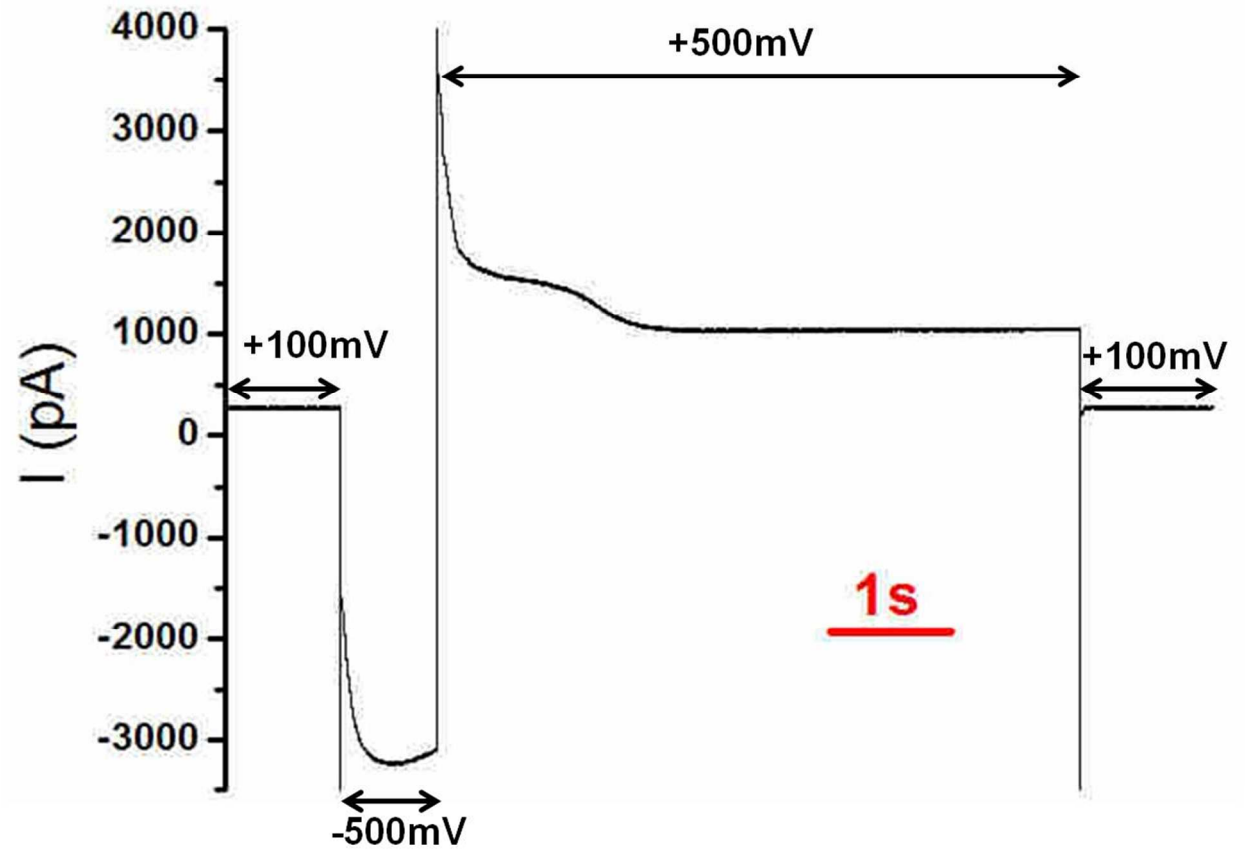


Supplementary Figure 1. SEM micrograph of representative nanopipette tips. Scanning electron microscopy (SEM) images of three different nanopipettes used for nanobiopsy.



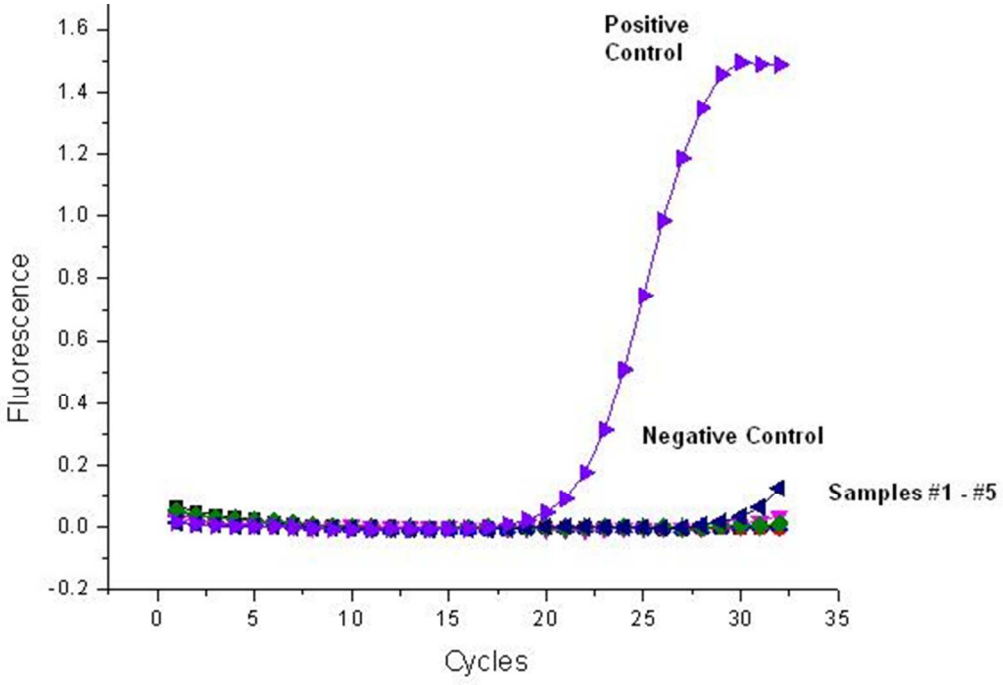
Supplementary Figure 2. Electrowetting in a nanopipette (Optical Characterization). Optical micrographs of a nanopipette filled with a 10mM solution of THATPBCl immersed in a PBS bath. A negative voltage of -500 mV causes the controlled ingress of aqueous solution into the nanopipette which can be reversed by switching the voltage to +500mV. Red squares are guides to eye for visualizing the aqueous solution aspirated into the nanopipette tip. See also Supp. Video 1.



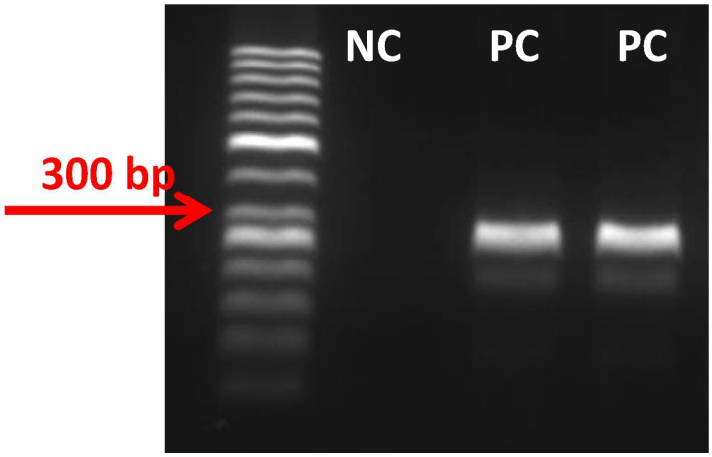
Supplementary Figure 3. Electrowetting in a nanopipette (Electrochemical Characterization). A nanopipette is filled with a 10mM solution of THATPBCl immersed in a PBS bath. The nanopipette is biased at +100mV to prevent aqueous solution from flowing into it. When the bias is switched to -500mV the measured ion current increases due to the entry of the aqueous solution (which has a higher conductance than the organic one) into the nanopipette barrel. When the voltage is switched to +500mV, the aspirated aqueous solution is expelled, resulting in a decrease in the measure ionic current. The curve obtained is the result 15 “aspiration-expulsion” cycles.

THATPBCl Salt Synthesis

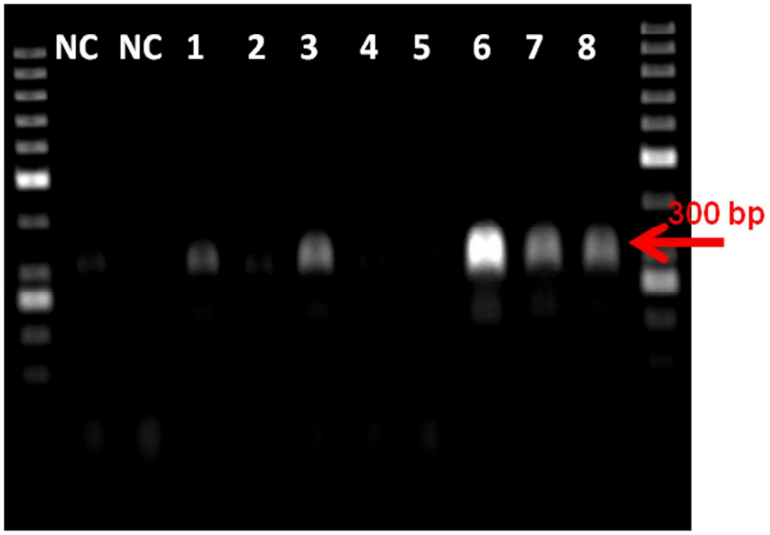
Tetrahexylammonium tetrakis(4-chlorophenyl)borate (THATPBCl) was synthesized by metathesis of tetrahexylammonium bromide (Aldrich, #263834) and potassium tetrakis(4-chlorophenyl)borate (Fluka, 60591). Both products were dissolved in a mixture of water/methanol (1:3) and re-crystallized in ethanol [1].



Supplementary Figure 4. Post biopsy analysis via qPCR. qPCR targeting GFP RNA from HeLa cells showing a positive control of total RNA from a ~1000 cells lysate (positive control, violet curve) and 5 negative controls (sample #1- #5) where no negative voltage was applied after cell penetration.



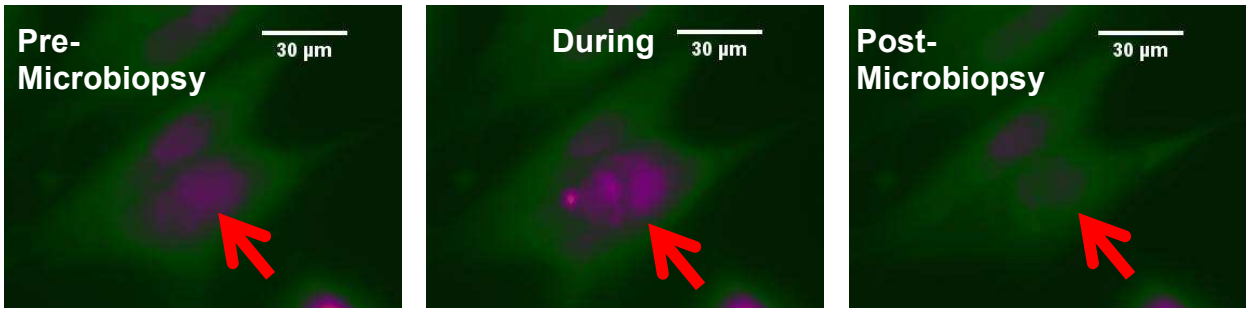
Supplementary Figure 5. Representative agarose gel stained with SYBR gold for fragment size determination (at expected size of ~250 base pairs) and polymerase chain reaction (PCR) amplification, following cDNA synthesis, for the GFP gene in GFP-HeLa cells (PC,positive control, ran in duplicate) and in human BJ fibroblasts (NC, negative control).



Supplementary Figure 6. Representative agarose gel stained with SYBR gold and polymerase chain reaction (PCR) amplification, following cDNA synthesis, for the GFP gene in GFP-HeLa cells for 8 nanobiopsies performed with 8 different nanopipettes.(NC,negative controls)

Calcium Imaging

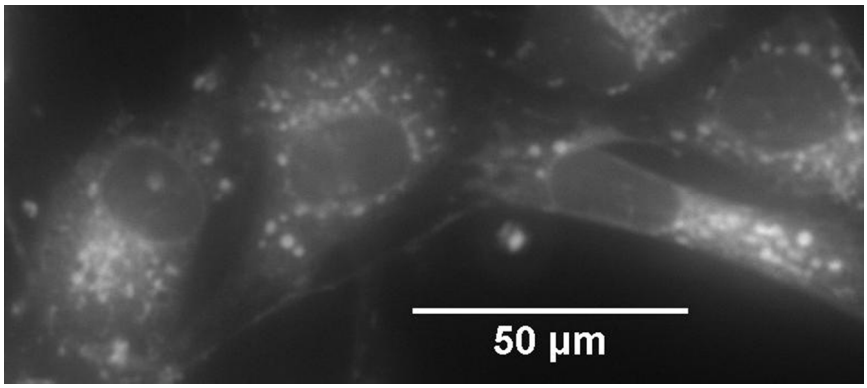
We tested the invasiveness of the nanobiopsy platform by monitoring [Ca^{2+}] before, during, and after the nanosurgical procedure. Human BJ fibroblasts were stained with 5μM Fluo-4AM (Invitrogen) in medium, described in cell culture section above, prior to experimentation. Cells were then incubated at 37°C for 30 minutes. Cells were washed twice with growth media before imaging.



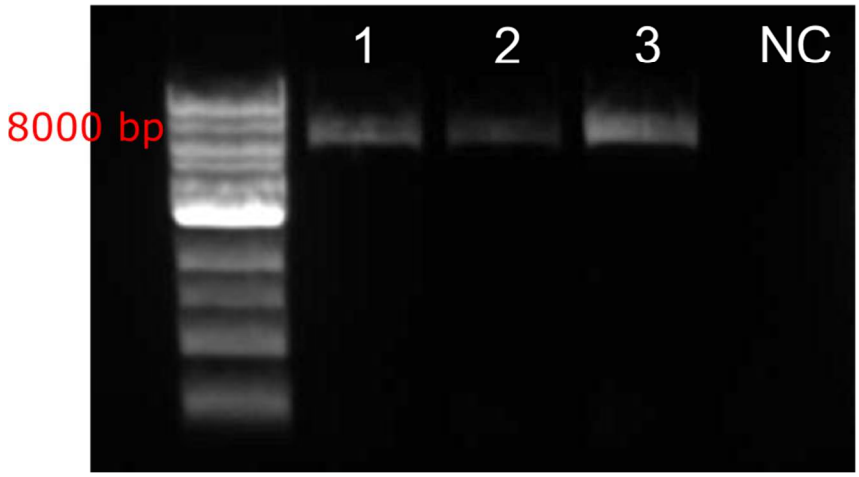
Supplementary Figure 7. Calcium imaging during microbiopsy procedure showing false color fluorescent micrographs of human BJ fibroblast cells stained with Fluo4 AM during microbiopsy. Red arrows indicate where the micropipette (5μm in diameter) penetrated the cell.

Visualization of the Mitochondria

Mitochondria were labeled with MitoTracker Green FM (Invitrogen) (excitation wavelength 490nm, emission wavelength 516 nm). MitoTracker Green was dissolved in dimethylsulfoxide (DMSO) at a concentration of 1mM and stored in -20°C. Adherent fibroblast cells were stained with a diluted solution of 1mM MitoTracker Green with a final concentration of 500nM in medium. Cells were incubated at 37°C for 30 minutes and washed twice. 1mL of medium was then added to cells for experimental work. Mitochondria are noticeably stained when observed under a fluorescent lamp.



Supplementary Figure 8. Fluorescent Micrograph of Human BJ Fibroblast cells stained with MitoTracker Green FM (Invitrogen).



Supplementary Figure 9. Representative 0.5% agarose gel stained with SYBR® Gold for fragment size determination (at expected size of 7800 base pairs) and polymerase chain reaction (PCR) amplification of mitochondrial DNA. Lanes 1-3, mitochondria biopsy on 3 individual human BJ fibroblast cells. Lane NC contains the negative control where no biopsy was performed (no input DNA).

Bioinformatics methods

RNA-Sequencing

Short reads were preprocessed to removed sequencing adapters and to trim off biased bases from the 5' end of both reads. Sequencing adapters were discarded using SeqPrep [https://github.com/jstjohn/SeqPrep]. 10 bases were removed from the 5' end to combat biases introduced by nonamer-based second strand synthesis during cDNA generation using fastx toolkit [http://hannonlab.cshl.edu/fastx_toolkit/]. Remaining reads were aligned to the UCSC human genome reference (hg19) and UCSC known gene annotations using Tophat v2.0.8b and Bowtie2 v2.1.0 [2, 3]. Duplicate reads were removed using SAMtools [4]. The number of tags corresponding to each mRNA annotation in UCSC known genes was counted using rseqc[5]. All mRNAs with at least 1 sequence tag were considered to be detected. A list of all detected mRNAs were used as input to the geneset enrichment analysis tool, GOSeq [6]. Overrepresented Gene Ontologies as detected by GOSeq were reported.

Mitochondrial-DNA Sequencing

Extracted mitochondrial DNA was amplified using long range PCR. The product was then sequenced using Illumina HiSeq. Resulting reads were aligned to a modified version of the UCSC human genome reference (hg19). The reference genome was modified by replacing the yoruban mitochondrial reference, chrM, with the revised Cambridge reference sequence, rCRS. Alignments were generated using Bowtie2 v2.1.0. PCR duplicates were removed using SAMtools. Average coverage depth was generated using Bedtools[7] . Heteroplasmic sites were detected using LoFreq[8] . Heteroplasmic sites with greater than 5% and less than 99% frequency were reported.

Video Supplement

Video 1: PBS Aspiration

Video 2: Nanobiopsy of Human BJ Fibroblast cells

References

1. Rimboud, M.; Charreteur, K.; Sladkov, V.; Elleouet, C.; Quentel, F.; L'Her, M. Effect of the Supporting Electrolytes on Voltammetry at Liquid/Liquid Microinterfaces Between Water and Nitrobenzene, 1,2-dichloroethane or 1,6-dichlorohexane. *J. Electroanal. Chem.* **2009**, 636, 53-59.
2. Kim, D.; Pertea, G.; Trapnell, C.; Pimentel, H.; Kelley, R.; Salzberg, S. TopHat2: Accurate Alignment of Transcriptomes in the Presence of Insertions, Deletions and Gene Fusions. *Genome Biology* **2013**, 14, R36.
3. Langmead, B.; Trapnell, C.; Pop, M.; Salzberg, S. Ultrafast and Memory-Efficient Alignment of Short DNA Sequences to the Human Genome. *Genome Biology* **2009**, 10, R25.
4. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; Subgroup, G. P. D. P. The Sequence Alignment/Map Format and SAMtools. *Bioinformatics* **2009**, 25, 2078-2079.
5. Wang, L.; Wang, S.; Li, W. RSeQC: Quality Control of RNA-seq Experiments. *Bioinformatics* **2012**, 28, 2184-2185.
6. Young, M.; Wakefield, M.; Smyth, G.; Oshlack, A. Gene Ontology Analysis for RNA-seq: Accounting for Selection Bias. *Genome Biology* **2010**, 11, R14.
7. Quinlan, A. R.; Hall, I. M. BEDTools: a Flexible Suite of Utilities for Comparing Genomic Features. *Bioinformatics* **2010**, 26, 841-842.
8. Wilm, A.; Aw, P. P. K.; Bertrand, D.; Yeo, G. H. T.; Ong, S. H.; Wong, C. H.; Khor, C. C.; Petric, R.; Hibberd, M. L.; Nagarajan, N. LoFreq: A Sequence-Quality Aware, Ultra-Sensitive Variant Caller for Uncovering Cell-Population Heterogeneity from High-Throughput Sequencing datasets. *Nucleic Acids Res.* **2012**, 40, 11189-11201.